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U.S. PATENT APPLICATION

for

Treatment of Helicobacter with Isothiocyanates

Inventors: Fahey, Jed W.

1 **Treatment of Helicobacter with Isothiocyanates**

2 **Inventor: Jed W. Fahey**

3 **STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR**
4 **DEVELOPMENT**

5 Part of the work performed during development of this invention
6 utilized U.S. Government funds. The U.S. Government has certain rights in
7 this invention.

8 **BACKGROUND OF THE INVENTION**

9 **Field of the Invention**

10 The present invention relates to methods of preventing or inhibiting
11 the growth of *Helicobacter* through the use of a composition that comprises
12 a glucosinolate, an isothiocyanate or a derivative or metabolite thereof. The
13 present invention also relates to methods of preventing or treating persistent
14 chronic gastritis, ulcers and/or stomach cancer in subjects at risk for, or in
15 need of treatment thereof.

16
17 **Background of the Invention**

18 Stomach cancer is the second most common form of cancer
19 worldwide. *Helicobacter pylori* is a microaerophilic, gram-negative bacterium
20 of cosmopolitan distribution that causes persistent chronic gastritis. Carriers
21 of *H. pylori* (in gastric mucosa) are at 3 to 6 times the risk for developing
22 stomach cancer (gastric adenocarcinoma and mucosa-associated lymphoid
23 tissue lymphoma) as non-carriers (J. Danesh *et al.*, *Cancer Surveys* , 33:263-
24 289 (1999); D. Forman *et al.*, *Br Med Bull*, 54:71-78 (1998); S. Hansen *et*
25 *al.*, *Scand J Gastroenterol*, 34:353-360 (1999) ; J-Q Huang *et al.*,

1 *Gastroenterology*, 114:1169-1179(1998)). *H. pylori* causes inflammation of
2 stomach tissue in carriers, resulting in increased blood flow, swelling and
3 irritation. Inflammation of the lower part of the stomach leads to ulcers in
4 about 10% of carriers. Inflammation of the upper part of the stomach leads
5 to impaired acid secretion and ultimate die-off of acid-producing cells and
6 leads to reduced stomach function and ultimately to cancer.

7 *Helicobacter pylori* was only first described following its cultivation
8 from human gastric biopsy specimens in 1982 (JR Warren et al., *Lancet*,
9 (1983), 1:1273-1275; BJ Marshall et al., *Microbios Lett.* (1984), 25:83-88).
10 Since then, as many as 26 related *Helicobacter* species have been described
11 colonizing the mucosal surfaces of humans and other animals (JV Solnick,
12 DB Schauer, *Clin Microbiol Rev*, (2001), 14:59-97). These organisms not
13 only colonize gastric tissues of mammals, but are found in the intestinal tract
14 and the liver of birds, as well as in mammals including humans, mice, ferrets,
15 gerbils, dogs and cats. They have been implicated as agents responsible for
16 inflammation, and in malignant transformation in immunocompetent hosts as
17 well as immunocompromised humans and animals. However, *H. pylori* is
18 now well-documented as one of the most prevalent human pathogens
19 worldwide (RM Genta et al., *Virchows Arch*, 425:339-347 (1994)), and the
20 causal agent for most gastric and duodenal ulcers, as well as a risk factor for
21 the development of gastric cancer (J Danesh, *Cancer Surveys*, 33:263-289
22 (1999)). The human stomach is the only known natural reservoir for *H.*
23 *pylori*, although many mammalian species can be infected by related species.
24 Antibiotic therapy aimed at eradication of *H. pylori* (e.g. amoxycillin and
25 clarithromycin plus the H₂ inhibitor omeprazol for 10-14 days) is now
26 recommended for infected patients who have verified peptic ulcerations of
27 the stomach or duodenum or who have gastric mucosa-associated lymphoid

1 tissue lymphomas, and cure rates are on the order of 90% (*Helicobacter*
2 Foundation, "Treatment of *Helicobacter pylori*, p. 1-5 (1998)). However, a
3 complex antibiotic therapy as described above may not be available in
4 developing countries, where *H. pylori* infection rates can be as high as 70%
5 of the population.

6 Thus a need exists for an economical dietary supplement, food or
7 pharmaceutical that will naturally inhibit the growth and/or infection rates of
8 *H. pylori*, both in the lumen of the stomach and within gastric epithelial cells
9 where *H. pylori* may serve as a low-level, chronic reservoir for re-infection.
10 This inhibition of eradication can in turn reduce the incidence of ulcers and
11 stomach cancer or prevent reinfection of *H. pylori*.

12 SUMMARY OF THE INVENTION

13 The present invention relates to a method of treating a subject having
14 a *Helicobacter* infection, comprising administering to the subject an
15 antibacterially effective amount of a composition that comprises a
16 glucosinolate, an isothiocyanate or a derivative thereof.

17 The present invention also relates to a method of preventing a
18 *Helicobacter* infection in a subject, comprising treating the subject with an
19 antibacterially effective amount of a composition that comprises a
20 glucosinolate, an isothiocyanate or a derivative thereof.

21 The present invention further relates to a method for inhibiting the
22 growth of *Helicobacter*, comprising administering an antibacterially effective
23 amount of an agent selected from the group consisting of a glucosinolate, an
24 isothiocyanate or a derivative thereof.

1 **BRIEF DESCRIPTION OF THE DRAWINGS**

2 N/A

3 **DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS**

4 The present invention relates to a method of treating a subject having
5 a *Helicobacter* infection, comprising administering to the subject an
6 antibacterially effective amount of a composition that comprises a
7 glucosinolate, an isothiocyanate or a derivative thereof.

8 *Helicobacter* is a gram-negative bacterium with polar flagella, using
9 oxygen as an electron acceptor, which cannot utilize carbohydrates as an
10 energy source. The *Helicobacter* genus is fully characterized in Versalovic,
11 *et al.*, *Manual of Clinical Microbiology*, 7th Ed., pp. 727-738 (1999) and
12 Perez-Perez, *et al.*, *Medical Microbiology*, 4th Ed., pp. 311-322 (1996), which
13 are incorporated herein by reference. *Helicobacter* is used interchangeably
14 with "*Helicobacter* sp" herein.

15 As used herein, the terms subject or patient are used interchangeably
16 and are used to mean any animal, preferably a mammal, including humans
17 and non-human primates. In one embodiment of the current invention the
18 subject having a *Helicobacter* infection is suffering from a peptic ulcer.
19 Peptic ulcers, as contemplated in the current invention include, but are not
20 limited to, circumscribed breaks in the continuity of the mucosal layer of the
21 gastrointestinal tract. These breaks in the continuity of the mucosal layer
22 can include breaks that extend below the epithelium, or breaks that do not
23 extend below the epithelium, sometime referred to as "erosions." The peptic
24 ulcers may be acute, or chronic. Further, peptic ulcers can be located in any
25 part of the gastrointestinal tract that is exposed to acid-pepsin gastric juice,

1 including the esophagus, stomach, duodenum, and after gastroenterostomy,
2 the jejunum.

3 In another embodiment of the current invention the subject having the
4 *Helicobacter* infection is suffering from, or at risk of developing, cancer of
5 the gastrointestinal tract. As stated previously, the portions of the
6 gastrointestinal tract where cancer may be present are any areas where the
7 tract is exposed to acid-pepsin gastric juice, including the esophagus,
8 stomach, duodenum, and after gastroenterostomy, the jejunum. As used
9 herein the term cancer is used as one of ordinary skill in the art would
10 recognize the term. Examples of cancers include, but are not limited to,
11 neoplasias (or neoplasms), hyperplasias, dysplasias, metaplasias,
12 hypertrophies. The neoplasms may be benign or malignant, and they may
13 originate from any cell type, including but not limited to epithelial cells of
14 various origin, muscle cells and endothelial cells.

15 The treatment envisioned by the current invention can be used for
16 patients with a pre-existing *Helicobacter* infection, or for patients pre-
17 disposed to a *Helicobacter* infection. Additionally, the method of the current
18 invention can be used to correct or compensate for cellular or physiological
19 abnormalities involved in conferring susceptibility to *Helicobacter* infection in
20 patients, and/or to alleviate symptoms of a *Helicobacter* infection in patients,
21 or as a preventative measure in patients.

22 As used herein, the phrase *Helicobacter* infection is used to mean an
23 interaction between *Helicobacter* and the host organism (subject). The
24 infections may be localized, meaning that the *Helicobacter* grows and
25 remains near the point of initial interaction. The infection may also be
26 generalized, where the *Helicobacter* may become more widespread beyond
27 the initial point of interaction, including spreading to the surrounding tissue

1 or organ and even being distributed and growing throughout the entire host
2 organism. As used herein, the term interaction (of a host and
3 microorganism) is used to mean a process where the *Helicobacter* grows in
4 or around a particular tissue. To illustrate, the *Helicobacter* is considered to
5 have infected the subject if the bacteria is able to penetrate the surface of
6 cells of a particular tissue and grow *within* the cells of the tissue. An
7 example of this type of infection includes, but is not limited to *Helicobacter*
8 penetrating and growing within the epithelial cells lining the lumen of the
9 stomach. Additionally, the *Helicobacter* can also be said to have infected
10 the host organism by growing extracellularly to the tissue cells.

11 The method of the current invention comprises administering an
12 antibacterially effective amount of a composition to treat a *Helicobacter*
13 infection. As used herein, "an antibacterially effective amount" is intended
14 to mean an amount effective to prevent, inhibit, retard or reverse the growth
15 of *Helicobacter*, and/or to reduce the number of viable *Helicobacter* cells
16 within the stomach or at a site of infection without excessive levels of side
17 effects. "Antibacterially effective amount" is also used to mean an amount
18 effective to kill, reduce or ameliorate any existing infections of *Helicobacter*
19 where the infection takes place prior to the administration of the
20 compositions used in the current invention. Thus as the current invention
21 contemplates, an antibacterially effective amount of the compositions of the
22 current invention can be used as a treatment to a pre-existing *Helicobacter*
23 infection. Effective amounts for use in these treatments can completely or
24 partially prevent a pre-existing infection from spreading to surrounding tissue
25 and beyond, and they can also be used to slow the growth and/or spread
26 rate of the *Helicobacter* in the subject. Furthermore, the antibacterially
27 effective amounts of the compositions used in the current invention can

1 prevent a *Helicobacter* infection in subjects. Another aspect of
 2 "antibacterially effective amount," as used in the current invention, means
 3 that the compositions administered to the subject are capable of preventing
 4 or reducing the cellular or physiological damage to the infected or
 5 surrounding tissue, caused by the toxins produced by the *Helicobacter*. In
 6 still another aspect, the phrase antibacterially effective amount can be used
 7 to mean an amount of the administered composition that can reduce or
 8 prevent the formation or efficacy of the virulence of the *Helicobacter*. By
 9 virulence is meant the ability of the *Helicobacter* to combat the host
 10 organism's or cell's natural defenses to the *Helicobacter* infection.

11 The method of treating a subject having a *Helicobacter* infection
 12 involves administration of compositions to the subjects. As used herein,
 13 composition can mean a pure compound, agent or substance or a mixture of
 14 two or more compounds, agents or substances. As used herein, the term
 15 agent, substance or compound is intended to mean a protein, nucleic acid,
 16 carbohydrate, lipid, polymer or a small molecule, such as a drug.

17 The compositions for use in the current invention comprise
 18 isothiocyanates, glucosinolates or derivatives or metabolites thereof such as,
 19 but not limited to: nitriles, carbamates, thiocarbamates, thiocyanates. As
 20 used herein derivatives include metabolites and/or analogs of isothiocyanates
 21 or glucosinolates. The term derivatives is used herein to encompass
 22 derivatives, analogs and metabolites of isothiocyanates or glucosinolates.
 23 Additionally, the compositions of the current invention also include
 24 combinations of different isothiocyanates, glucosinolates or derivatives
 25 thereof or their combination with other therapeutic moieties or agents.
 26 Isothiocyanates are compounds containing the isothiocyanate (-NCS) moiety
 27 and are easily identifiable by one of ordinary skill in the art. An example of

1 an isothiocyanate includes, but is not limited to sulforaphane or its analogs.
2 The description and preparation of isothiocyanate analogs is described in
3 United States Reissue Patent 36,784, and is hereby incorporated by
4 reference in its entirety. In a preferred embodiment, the sulforaphane
5 analogs used in the present invention include 6-isothiocyanato-2-hexanone,
6 exo-2-acetyl-6-isothiocyanatonorbornane, exo-2-isothiocyanato-6-
7 methylsulfonylnorbornane, 6-isothiocyanato-2-hexanol, 1-isothiocyanato-4-
8 dimethylphosphonylbutane, exo-2-(1'-hydroxyethyl)-5-
9 isothiocyanatonorbornane, exo-2-acetyl-5-isothiocyanatonorbornane, 1-
10 isothiocyanato-5-methylsulfonylpentane, cis-3-
11 (methylsulfonyl)cyclohexylmethylisothiocyanate and trans-3-
12 (methylsulfonyl)cyclohexylmethylisothiocyanate. Other isothiocyanates also
13 include, but are not limited to, conjugates of isothiocyanates, which include,
14 among others, glutathione-, cysteinylglycine-, cysteinyl-, and N-
15 acetylcysteine- conjugates.

16 Glucosinolates, which are well-known in the art, are precursors to
17 isothiocyanates. Examples of glucosinolates include, but are not limited to,
18 glucoraphanin, glucoerysolin, glucoerucin, glucoiberin, glucoalyssin,
19 glucoberteroin, glucoibererin, glucocheirolin, glucoraphenin, 5-
20 methylsulfinylpentyl glucosinolate, 6-methylsulfinylhexyl glucosinolate, 7-
21 methylsulfinylheptyl glucosinolate, 8-methylsulfinyloctyl glucosinolate, 9-
22 methylsulfinylnonyl glucosinolate, 10-methylsulfinyldecyl glucosinolate,
23 phenylethyl glucosinolate, 4-(α -L-rhamnopyranosyloxy)benzyl glucosinolate,
24 3-(α -L-rhamnopyranosyloxy)benzyl glucosinolate, 2-(α -L-
25 rhamnopyranosyloxy)benzyl glucosinolate, 4-(4'-O-acetyl- α -L-
26 rhamnopyranosyloxy)benzyl glucosinolate as well as those reviewed in Table
27 1 of Fahey *et al.*, *Phytochemistry*, 56:5-51 (2001) and corrigenda thereto,

the entire contents of which are incorporated herein by reference, and the products of their myrosinase-catalyzed hydrolysis (e.g. their cognate isothiocyanates, thiocyanates, nitriles, carbamates and thiocarbamates). Glucosinolates are easily recognizable and appreciated by one of ordinary skill in the art and are reviewed in Fahey *et al.*, *Phytochemistry*, 56:5-51 (2001) and corrigenda thereto, the entire contents of which are hereby incorporated by reference.

In one embodiment of the current invention, the isothiocyanate for use in the current invention is sulforaphane, or a derivative thereof. In a further embodiment, the isothiocyanate is sulforaphane.

Sulforaphane (4-methylsulfinylbutyl isothiocyanate or (-)-1-isothiocyanato-4(R)-(methylsulfinyl) butane) and sulforaphene (4-methylsulfinylbutenyl isothiocyanate) and their cognate glucosinolates (glucoraphanin and glucoraphenin, respectively), are known to be produced by plants, such as hoary cress, radish and other plants (Mislow *et al.*, *J. Am. Chem. Soc.*, 87:665-666 (1965); Schmid *et al.*, *Helvet. Chim. Acta*, 31:1017-1028 (1942); Hansen *et al.*, *Acta Chem. Scand. Ser., B* 28:418-424 (1974)). For the purposes of the present invention, they can be isolated from plants or synthesized. Bertoin, allysin, erucin, erysolin, iberiverin, iberin, and cheirolin can also be isolated from plants; these compounds appear to be less active as inducers than sulforaphane and sulforaphene, at least in cell culture.

Other synthetic analogues include compounds with sulfur-containing-, olefinic, aliphatic, and multiply glycosylated- side chains.

Other analogues of sulforaphane can be used which are not specifically shown. The relative ability of the compound to inhibit or prevent the growth of *Helicobacter*, or treat subjects with *Helicobacter* infections can

1 be assessed as taught below, either by testing inhibition in cell lines, or in
2 whole animals.

3 Provided by the present invention are food products which have been
4 supplemented with a composition or agent of the present invention. The
5 compositions or agents used as food supplements should contain
6 isothiocyanates, glucosinolates or derivatives thereof. The supplement may
7 be isolated from plants or synthesized. Also provided by the present
8 invention are foods and/or plants that contain high levels of glucosinolates or
9 isothiocyanates. Examples of plants that contain glucosinolates or
10 isothiocyanates include, but are not limited to, *Brassicaceae* (*Cruciferae*),
11 *Moringaceae* and *Resedaceae*, which collectively include, but are not limited
12 to, broccoli, broccoli sprouts, Brussels sprouts, cabbage, cauliflower,
13 cauliflower sprouts, daikon, horseradish, kale, mustard seed, radish, wasabi,
14 horseradish tree (*Moringa oleifera*), cabbage tree (*M. stenopetala*),
15 mignonette (*Reseda odorata*), dyer's rocket (*R. luteola*). Other families of
16 plants that contain glucosinolates include, but are not limited to, *Bataceae*,
17 *Bretschneideraceae*, *Capparaceae*, *Caricaceae*, *Euphorbiaceae*,
18 *Gyrostemonaceae*, *Limnanthaceae*, *Pentadiplandraceae*, *Phytolaccaceae*,
19 *Pittosporaceae*, *Salvadoraceae*, *Tovariaceae* and *Tropaeolaceae*. These high
20 levels may occur naturally or plants may be bred to contain high levels or
21 glucosinolates or isothiocyanates.

22 Glucosinolates and/or isothiocyanates can be purified from seed or plant
23 extracts by methods well known in the art. (See Fenwick *et al.*, *CRC Crit.*
24 *Rev. Food Sci. Nutr.* 18: 123-201 (1983) and Zhang *et al.*, *Proc. Natl Acad.*
25 *Sci. USA* 89: 2399-2403 (1992)). Purified or partially purified glucosinolate(s)
26 or isothiocyanate(s) can be added to food products as a supplement. The dose
27 of glucosinolate and/or isothiocyanate added to the food product preferably is

1 in the range of 1 μ mol to 1,000 μ mol per serving. However, the dose of
2 glucosinolate and/or isothiocyanate supplementing the food product can be
3 higher.

4 The selection of plants having high levels of glucosinolates or
5 isothiocyanates in sprouts, seeds or other plant parts can be incorporated into
6 *Brassica (Crucifer)* breeding programs. In addition, these same breeding
7 programs can include the identification and selection of cultivars that have high
8 levels of glucosinolates or isothiocyanates. Strategies for the crossing,
9 selection and breeding of new cultivars of *Brassicaceae (Cruciferae)* are well
10 known to the skilled artisan in this field. (*Brassica Crops and Wild Allies:*

11 *Biology & Breeding*; S. Tsunoda *et al.* (eds), Japan Scientific Societies Press,
12 Tokyo pp. 354 (1980); *Biology of Brassica Coenospecies*; C. Gomez-Campo
13 (ed), Elsevier, Amsterdam p. 489 (1999)). Progeny plants are screened for

14 high levels of glucosinolates or isothiocyanates produced at specific plant
15 developmental stages. Plants carrying the trait of interest are identified and
16 the characteristic intensified or combined with other important agronomic
17 characteristics using breeding techniques well known in the art of plant
18 breeding.

19 In one embodiment of the current invention, the composition used in
20 the method of treating a *Helicobacter* infection can be in the form of a food,
21 food supplement, a dietary supplement or food additive.

22 In one embodiment of the current invention, the composition
23 administered to the subject is a pharmaceutical composition. Further, the
24 pharmaceutical composition can be administered orally, nasally, parenterally,
25 intrasystemically, intraperitoneally, topically (as by drops or transdermal
26 patch), buccally, or as an oral or nasal spray. The term "parenteral," as used
27 herein, refers to modes of administration which include intravenous,

1 intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular
2 injection and infusion. The pharmaceutical compositions as contemplated by
3 the current invention may also include a pharmaceutically acceptable carrier.

4 By "pharmaceutically acceptable carrier" is intended, but not limited
5 to, a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material
6 or formulation auxiliary of any type.

7 A pharmaceutical composition of the present invention for parenteral
8 injection can comprise pharmaceutically acceptable sterile aqueous or
9 nonaqueous solutions, dispersions, suspensions or emulsions as well as
10 sterile powders for reconstitution into sterile injectable solutions or
11 dispersions just prior to use. Examples of suitable aqueous and nonaqueous
12 carriers, diluents, solvents or vehicles include water, ethanol, polyols (such
13 as glycerol, propylene glycol, polyethylene glycol, and the like),
14 carboxymethylcellulose and suitable mixtures thereof, vegetable oils (such as
15 olive oil), and injectable organic esters such as ethyl oleate. Proper fluidity
16 can be maintained, for example, by the use of coating materials such as
17 lecithin, by the maintenance of the required particle size in the case of
18 dispersions, and by the use of surfactants.

19 The compositions of the present invention can also contain adjuvants
20 such as, but not limited to, preservatives, wetting agents, emulsifying
21 agents, and dispersing agents. Prevention of the action of microorganisms
22 can be ensured by the inclusion of various antibacterial and antifungal
23 agents, for example, paraben, chlorobutanol, phenol, sorbic acid, and the
24 like. It can also be desirable to include isotonic agents such as sugars,
25 sodium chloride, and the like. Prolonged absorption of the injectable
26 pharmaceutical form can be brought about by the inclusion of agents which
27 delay absorption such as aluminum monostearate and gelatin.

1 In some cases, to prolong the effect of the drugs, it is desirable to
2 slow the absorption from subcutaneous or intramuscular injection. This can
3 be accomplished by the use of a liquid suspension of crystalline or
4 amorphous material with poor water solubility. The rate of absorption of the
5 drug then depends upon its rate of dissolution which, in turn, can depend
6 upon crystal size and crystalline form. Alternatively, delayed absorption of a
7 parenterally administered drug form is accomplished by dissolving or
8 suspending the drug in an oil vehicle.

9 Injectable depot forms are made by forming microencapsule matrices
10 of the drug in biodegradable polymers such as polylactide-polyglycolide.

11 Depending upon the ratio of drug to polymer and the nature of the particular
12 polymer employed, the rate of drug release can be controlled. Examples of
13 other biodegradable polymers include poly(orthoesters) and poly(anhydrides).

14 Depot injectable formulations are also prepared by entrapping the drug in
15 liposomes or microemulsions which are compatible with body tissues.

16 The injectable formulations can be sterilized, for example, by filtration
17 through a bacterial-retaining filter, or by incorporating sterilizing agents in the
18 form of sterile solid compositions which can be dissolved or dispersed in
19 sterile water or other sterile injectable medium just prior to use.

20 Solid dosage forms for oral administration include, but are not limited
21 to, capsules, tablets, pills, powders, and granules. In such solid dosage
22 forms, the active compounds are mixed with at least one item
23 pharmaceutically acceptable excipient or carrier such as sodium citrate or
24 dicalcium phosphate and/or a) fillers or extenders such as starches, lactose,
25 sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example,
26 carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and
27 acacia, c) humectants such as glycerol, d) disintegrating agents such as

1 agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain
2 silicates, and sodium carbonate, e) solution retarding agents such as paraffin,
3 f) absorption accelerators such as quaternary ammonium compounds, g)
4 wetting agents such as, for example, acetyl alcohol and glycerol
5 monostearate, h) absorbents such as kaolin and bentonite clay, and i)
6 lubricants such as talc, calcium stearate, magnesium stearate, solid
7 polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the
8 case of capsules, tablets and pills, the dosage form can also comprise
9 buffering agents.

10 Solid compositions of a similar type can also be employed as fillers in
11 soft and hard filled gelatin capsules using such excipients as lactose or milk
12 sugar as well as high molecular weight polyethylene glycols and the like.

13 The solid dosage forms of tablets, dragees, capsules, pills, and
14 granules can be prepared with coatings and shells such as enteric coatings
15 and other coatings well known in the pharmaceutical formulating art. They
16 can optionally contain opacifying agents and can also be of a composition
17 that they release the active ingredient(s) only, or preferentially, in a certain
18 part of the intestinal tract, optionally, in a delayed manner. Examples of
19 embedding compositions which can be used include polymeric substances
20 and waxes.

21 The active compounds can also be in micro-encapsulated form, if
22 appropriate, with one or more of the above-mentioned excipients.

23 Liquid dosage forms for oral administration include, but are not limited
24 to, pharmaceutically acceptable emulsions, solutions, suspensions, syrups
25 and elixirs. In addition to the active compounds, the liquid dosage forms can
26 contain inert diluents commonly used in the art such as, for example, water
27 or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol,

1 isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl
2 benzoate, propylene glycol, 1,3-butylene glycol, dimethyl formamide, oils (in
3 particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame
4 oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid
5 esters of sorbitan, and mixtures thereof.

6 Besides inert diluents, the oral compositions can also include adjuvants
7 such as wetting agents, emulsifying and suspending agents, sweetening,
8 flavoring, and perfuming agents.

9 Suspensions, in addition to the active compounds, can contain
10 suspending agents as, for example, ethoxylated isostearyl alcohols,
11 polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose,
12 aluminum metahydroxide, bentonite, agar-agar, and tragacanth, and mixtures
13 thereof.

14 Alternatively, the composition can be pressurized and contain a
15 compressed gas, such as nitrogen or a liquefied gas propellant. The liquefied
16 propellant medium and indeed the total composition is preferably such that
17 the active ingredients do not dissolve therein to any substantial extent. The
18 pressurized composition can also contain a surface active agent. The
19 surface active agent can be a liquid or solid non-ionic surface active agent or
20 can be a solid anionic surface active agent. It is preferred to use the solid
21 anionic surface active agent in the form of a sodium salt.

22 The compositions of the present invention can also be administered in
23 the form of liposomes. As is known in the art, liposomes are generally
24 derived from phospholipids or other lipid substances. Liposomes are formed
25 by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an
26 aqueous medium. Any non-toxic, physiologically acceptable and
27 metabolizable lipid capable of forming liposomes can be used. The present

1 compositions in liposome form can contain, in addition to the compounds of
2 the invention, stabilizers, preservatives, excipients, and the like. The
3 preferred lipids are the phospholipids and the phosphatidyl cholines
4 (lecithins), both natural and synthetic. Methods to form liposomes are
5 known in the art (see, for example, Prescott, Ed., *Meth. Cell Biol.* 14:33 et
6 seq (1976)).

7 One of ordinary skill will appreciate that effective amounts of the
8 agents of the invention can be determined empirically and can be employed
9 in pure form or, where such forms exist, in pharmaceutically acceptable salt,
10 ester or prodrug form. The agents can be administered to a subject, in need
11 of treatment of a *Helicobacter* infection, as pharmaceutical compositions in
12 combination with one or more pharmaceutically acceptable excipients. It will
13 be understood that, when administered to a human patient, the total daily
14 usage of the agents or composition of the present invention will be decided
15 by the attending physician within the scope of sound medical judgement.
16 The specific therapeutically effective dose level for any particular patient will
17 depend upon a variety of factors: the type and degree of the cellular or
18 physiological response to be achieved; activity of the specific agent or
19 composition employed; the specific agents or composition employed; the
20 age, body weight, general health, sex and diet of the patient; the time of
21 administration, route of administration, and rate of excretion of the agent;
22 the duration of the treatment; drugs used in combination or coincidental with
23 the specific agent; and like factors well known in the medical arts. For
24 example, it is well within the skill of the art to start doses of the agents at
25 levels lower than those required to achieve the desired therapeutic effect and
26 to gradually increase the dosages until the desired effect is achieved.

1 Dosing can also be arranged in a patient specific manner to provide a
2 predetermined concentration of the agents in the blood, as determined by
3 techniques accepted and routine in the art. Thus patient dosaging can be
4 adjusted to achieve regular on-going blood levels, as measured by HPLC, on
5 the order of from 50 to 1000 ng/ml.

6 It will be readily apparent to one of ordinary skill in the relevant arts
7 that other suitable modifications and adaptations to the methods and
8 applications described herein can be made without departing from the scope
9 of the invention or any embodiment thereof.

10 In one embodiment of the current invention, the *Helicobacter* infection
11 from which the subject is suffering is *Helicobacter pylori*.

12 In another embodiment, the methods of the current invention may
13 further comprise administering an antibiotic, an antibiotic regimen or another
14 drug to the subject or *Helicobacter*. The current pharmaceutical regimen for
15 treating *H. pylori* includes antibiotic therapy. As used herein, the phrase
16 "antibiotic" or "antibiotic therapy" is used as one of skill in the art would
17 recognize such terms. Antibiotics for use in combination with the
18 compositions or agents in the current invention include, but are not limited
19 to, amoxycillin and clarithromycin. Other drugs that may be used in
20 combination with the current invention include, but are not limited to,
21 omeprazol.

22 The present invention also relates to a method of preventing a
23 *Helicobacter* infection in a subject, comprising treating said subject with an
24 antibacterially effective amount of a composition that comprises a
25 glucosinolate, an isothiocyanate or a derivative thereof. Preferably, the
26 method of preventing *Helicobacter* infection is performed on *Helicobacter*
27 *pylori*.

1 As used herein the method of preventing a *Helicobacter* infection may
2 be performed on subjects that have had previous infections, or on subjects
3 with no history of *Helicobacter* infection.

4 In one embodiment of the current invention, the compositions used to
5 prevent *Helicobacter* infection in a subject comprise sulforaphane or a
6 derivative thereof. In a further embodiment, the composition is
7 sulforaphane.

8 In another embodiment of the current invention, the composition used
9 to prevent *Helicobacter* is a food, food supplement, dietary supplement or a
10 food additive. In still another embodiment, the composition is a
11 pharmaceutical composition. Preferably, the pharmaceutical composition is
12 administered orally.

13 The current invention also relates to a method for inhibiting the
14 growth of *Helicobacter*, comprising administering to said *Helicobacter* an
15 antibacterially effective amount of an agent selected from the group
16 consisting of a glucosinolate, a isothiocyanate or a derivative thereof.
17 Preferably, the *Helicobacter* is *Helicobacter pylori*.

18 As used herein, inhibition of growth is used to mean growth under *in*
19 *vitro*, *in vivo* or *in situ* conditions. Furthermore, inhibition of growth is used
20 to mean the process where the bacteria cells stop or slow their rate of
21 mitosis or normal metabolic processes. Inhibition of growth can also mean
22 cell death. The various forms and signs of cell death are obvious to those
23 skilled in the art, but examples of cell death include, but are not limited to,
24 programmed cell death (i.e., apoptosis), gradual death of the cells as occurs
25 in diseased states (i.e., necrosis), and more immediate cell death such as
26 acute toxicity. The inhibition of growth of *Helicobacter* for which the current

1 invention provides can be a complete or partial inhibition of growth or a
2 complete or partial causation of cell death.

3 In one embodiment of the current invention, the compositions used to
4 inhibit the growth of *Helicobacter* infection in a subject comprise
5 sulforaphane or a derivative thereof. In a further embodiment, the
6 composition is sulforaphane.

7 In another embodiment of the current invention, the composition used
8 to inhibit the growth of *Helicobacter* is a food, food supplement, dietary
9 supplement or a food additive. In still another embodiment, the composition
10 is a pharmaceutical composition. Preferably, the pharmaceutical composition
11 is administered orally.

12 In another embodiment, the compositions of the current invention may
13 be combined with antibiotics or other drugs to prevent the growth of
14 *Helicobacter*.

15 The current invention also relates to a method of identifying an agent
16 that modulates the growth of *Helicobacter* comprising treating *Helicobacter*
17 with said agent and assaying for growth of said *Helicobacter*; treating said
18 *Helicobacter* with a known modulator of *Helicobacter* growth and assaying
19 for growth of said *Helicobacter*, wherein said known modulator of
20 *Helicobacter* growth is selected from the group consisting of a glucosinolate,
21 an isothiocyanate and a derivative thereof; and comparing the levels of
22 *Helicobacter* growth in (a) and (b) to determine if said agent modulates said
23 growth of *Helicobacter*. Preferably, the method of screening agents that
24 modulate the growth of *Helicobacter* is used to screen agents that modulate
25 the growth of *Helicobacter pylori*.

26 In one embodiment of the current invention, the method of identifying
27 an agent that modulates the growth *Helicobacter* is performed on a single

1 population of cells, and (b) is performed on the identical population after the
 2 agent in (a) is removed. In another embodiment of the invention, the method
 3 of identifying an agent that modulates the growth *Helicobacter* is performed
 4 on two nearly identical populations of cells, under the same conditions,
 5 where (a) is performed on one population and (b) is performed on another
 6 population, and (c) is a comparison of the levels of the growth *Helicobacter*
 7 between the two populations of cells. Preferably, the methods of identifying
 8 growth modulators of *Helicobacter* are performed on *Helicobacter pylori*.

9 In another embodiment, of the current invention, the method of
 10 identifying an agent that modulates the growth *Helicobacter* is performed on
 11 cells other than *Helicobacter* cells, that have been infected with the
 12 *Helicobacter* prior to the assay. The *Helicobacter* may be present inside these
 13 other cells or it may be present around, or near, the cells. Examples of
 14 situations where the *Helicobacter* may be present in or around the other cell
 15 types include, but are not limited to, co-culturing cells with *Helicobacter*,
 16 allowing the *Helicobacter* to infect the other cell types prior to performing
 17 the assay. The other cells can be prokaryotic or eukaryotic, but preferably
 18 eukaryotic, and even more preferably animal cells. The animal cells for use
 19 in the current invention can be any type of cell found in an animal, including,
 20 but not limited to, epithelial, neuronal, endothelial and muscle cells.

21 In an additional embodiment, the methods of identifying agents that
 22 modulate the growth of *Helicobacter* can be carried out on cells that are in
 23 culture, i.e. *in vitro*, or in cells occurring *in situ* or *in vivo*. The cells may be
 24 part of a tissue or a whole organ. As used herein, the term tissue is used to
 25 mean a tissue as one of ordinary skill in the art would understand it to mean.
 26 As envisioned in the current application, tissue is also used to mean
 27 individual or groups of cells, or cell cultures, of a bodily tissue or fluid (e.g.

blood cells). Furthermore, the tissue may be within a subject, or biopsied or removed from a subject. The tissue may also be a whole or any portion of a bodily organ. Additionally, the tissue may be "fresh" in that the tissue would be recently removed from a subject without any preservation steps between the excision and the methods of the current invention. The tissue may also have been preserved by such standard tissue preparation techniques including, but not limited to, freezing, quick freezing, paraffin embedding and tissue fixation, prior to application of the methods of the current invention. Furthermore, the tissue may also be a xenograft or a syngraft on or in another host animal.

The types of agents or compounds which can be envisioned are limited only by their ability to modulate the growth of *Helicobacter*. The agents of the present invention may be identified and/or prepared according to any of the methods and techniques known to those skilled in the art. Preferably, the agents of the present invention are selected and screened at random or rationally selected or designed using chemical modeling techniques, based on structure-activity relationships (SAR).

For random screening, candidate agents are selected at random and assayed for their ability to modulate the growth of *Helicobacter*. Any of the suitable methods and techniques known to those skilled in the art may be employed to assay candidate agents.

For rational selection or design, the agent is selected based on the chemical structure of known modulators of the growth of *Helicobacter*. Any of the suitable methods and techniques, or modifications thereof, known to those skilled in the art may be employed for rational selection or design. For example, one skilled in the art can readily adapt currently available

1 procedures to generate peptides, pharmaceutical agents and the like capable
2 of modulating the growth of *Helicobacter*.

3 In another embodiment, the known modulators for use in the assay of
4 the current invention are isothiocyanate, sulforaphane, sulforaphene,
5 erysolin, erucin, iberin, alysin, berteroin, iberiverin, cheirolin, 5-
6 methylsulfinylpentyl isothiocyanate, 6-hexylsulfinyl isothiocyanate, 7-
7 methylsulfinylheptyl isothiocyanate, 8-methylsulfinyloctyl isothiocyanate, 9-
8 methylsulfinylnonyl isothiocyanate, 10-methylsulfinyldecyl isothiocyanate,
9 phenylethyl isothiocyanate, 4-(α -L-rhamnopyranosyloxy)benzyl
10 isothiocyanate, 3-(α -L-rhamnopyranosyloxy)benzyl isothiocyanate, 2-(α -L-
11 rhamnopyranosyloxy)benzyl isothiocyanate, 4-(4'-O-acetyl- α -L-
12 rhamnopyranosyloxy)benzyl isothiocyanate or a derivative thereof. The
13 isothiocyanates, glucosinolates or derivatives thereof for use in the methods
14 of identifying modulators of *Helicobacter* growth have been described
15 previously herein. In still another embodiment, the known modulator is
16 sulforaphane.
17 The following Examples serve only to illustrate the invention, and
18 should not be construed, in any way, to limit the invention.

1 **Examples**2 Example 1

3 A preparation of broccoli sprout extract was delivered to *H. pylori*
 4 growth medium both with and without fetal calf serum (FCS) which is
 5 reported to ameliorate the effects of some antibiotics against *H. pylori*. The
 6 first column below indicates the dilution of broccoli sprout extract used, the
 7 second column indicates the actual sulforaphane concentration in test article.

Test Article		<u>Sulforaphane effects on <i>H. pylori</i> strain 26695 growth</u>	
<u>Dilution</u>	<u>Sulforaphane</u>	<u>Test Medium</u>	<u>Test Medium</u>
<u>(1/x)</u>	<u>Conc.</u>	<u>(Serum-Free)</u>	<u>(+ 1 % FCS)</u>
100	1940 μ M	Complete inhibition	complete inhibition
500	388 μ M	Complete inhibition	complete inhibition
2,500	78 μ M	complete inhibition	complete inhibition
12,500	16 μ M	complete inhibition	>4 log reduction
62,500	3.1 μ M	slight suppression	> 1 log reduction

8 (3.2 μ M \approx 0.57 ppm sulforaphane)

9 Example 2

10 To assess the ability of sulforaphane to inhibit the growth of *H. pylori*,
 11 compared to traditional antibiotic therapies, several strains of *H. pylori* were
 12 cultured in the presence or absence of sulforaphane or antibiotics, and the
 13 minimum inhibitory concentrations (MIC) of each were compared. The
 14 antibiotics against which sulforaphane was compared were amoxycillin,
 15 clarithromycin and metronidazole. The data below demonstrate that
 16 sulforaphane is as effective, if not more effective, as traditional antibiotics in
 17 inhibiting the growth of *H. pylori*.

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Table 1
Bacteriostatic activity of sulforaphane against
amoxicillin/clarithromycin/metronidazole - susceptible strains of *Helicobacter*
***pylori* (n = 32)**

Strain no	MIC (μ g/ml) of			
	Amoxicillin	Clarithromycin	Metronidazole	Sulforaphane
1	0.06	0.06	1	2
2	0.06	0.125	1	4
3	0.06	0.125	1	2
4	0.06	0.06	1	4
5	0.06	0.06	4	4
6	0.06	0.06	0.06	0.06
7	0.06	0.06	0.06	0.06
8	0.06	0.06	0.5	0.5
9	0.125	0.06	1	2
10	0.06	0.06	0.5	0.06
11	0.06	0.06	0.25	2
12	0.06	0.06	1	4
13	0.06	0.06	1	4
14	0.06	0.06	0.06	0.06
15	0.06	0.06	0.06	0.06
16	0.06	0.06	0.5	1
17	0.06	0.06	1	0.5
18	0.06	0.06	1	0.5
19	0.06	0.06	0.125	2
20	0.06	0.06	0.125	0.5

21	0.125	0.06	0.125	0.5
22	0.06	2	0.125	0.06
23	0.06	0.06	0.06	0.06
24	0.06	0.06	0.5	1
25	0.06	0.06	1	0.5
26	0.06	0.06	1	0.5
27	0.06	0.06	0.06	0.06
28	0.06	0.125	0.5	0.5
29	0.125	0.06	1	2
30	0.06	0.06	0.5	0.06
31	0.06	0.06	0.25	4
32	0.06	0.06	1	2

Table 2

**Bacteriostatic activity of sulforaphane against clarithromycin and/or
metronidazole - intermediate or resistant strains of *Helicobacter pylori*
(n = 15)**

Strain no	MIC (μ g/ml) of			
	Amoxicillin	Clarithromycin	Metronidazole	Sulforaphane
33	0.06	0.06	32	4
34	0.125	0.125	256	0.5
35	0.06	0.06	64	4
36	0.06	0.06	64	4
37	0.06	0.06	64	0.5
38	0.125	0.5	64	2
39	0.06	0.125	256	0.5

40	0.06	0.06	64	4
41	0.06	4	16	0.125
42	0.06	16	1	4
43	0.06	16	1	8
44	0.06	16	0.5	0.5
45	0.06	8	2	1
46	0.06	16	32	2
47	0.06	16	64	4

Table 3

Time course for Efficacy of Sulforaphane activity against *Helicobacter pylori*

Conc. of sulforaphane Tested	Time (h) at which 99.9% intracellular killing was observed for			
	HP 1* (MIC = 2 μ g/ml)	HP 2** ^a (MIC = 2 μ g/ml)	HP 3* (MIC = 4 μ g/ml)	HP 4** (MIC = 0.06 μ g/ml)
1x MIC	8	-	4	8
5x MIC	8	-	4	8
10x MIC	8	-	4	8
20x MIC	8	-	2	4

* tested in triplicate

** tested in duplicate - definitive results will be available next week

^a A less than 10,000-fold (99.9%) reduction in colony forming units (CFU) was observed with this strain. Actual reductions (\log_{10} CFU) for this strain follow:

Time (h)	1x MIC	5x MIC	10x MIC	20x MIC
2	0	0	0	- 0.12
4	- 0.79	- 0.90	- 1.17	- 1.30
8	- 0.90	- 0.90	- 1.30	- 1.40
24	- 1.18	- 1.20	- 1.34	- 1.40
48	- 1.20	- 1.20	- 1.40	- 1.40

1 Example 3

2 Bacteria are grown in broth cultures to log phase, collected by
3 centrifugation and resuspended in PBS. Groups of animals (mice and gerbils)
4 are dosed with 10^9 CFU/ml of *H. pylori* in PBS, either by gavage (100 μ L
5 delivered via a round-end cannula, or by oral inoculation (delivery of 30-50
6 μ L of *H. pylori* in PBS via micropipet following the removal of access to food
7 and water for 3 to 6 hours). Animal groups are housed in microisolator
8 cages and handled by personnel wearing protective clothing. At various
9 time-points, animals are anesthetized with metaphane, exsanguinated by
10 cardiac puncture, and then sacrificed by cervical dislocation to assess
11 infection status. Infection status are measured by direct culture, histology,
12 and a rapid urease test that is highly indicative of *H. pylori* presence (Y.
13 Tokunaga *et al*, *J Gastroenterol Hepatol* 15:617-621 (2000)). *H. pylori* is
14 cultured from gastric mucosa on semi-solid culture medium with antibiotics
15 to inhibit the growth of contaminating organisms, and colony confirmation is
16 made based on colony morphology and microscopic examination. A
17 pathologist examines tissues for macroscopic signs of inflammation and/or
18 erosion, and microscopic analysis of fixed tissues is performed on paraffin
19 sections stained by the modified Giemsa or modified Steiner method and
20 graded on a 0-4 scale (RK Vartanian *et al.*, *Mod Pathol*, (1998), 11:72-78; O
21 Rotimi *et al.*, *J Clin Pathol*, 53:756-759 (2000)). These widely used

1 methods are initially used to optimize infection techniques, and to determine
2 which of the *H. pylori* strains will best colonize the animals to be used in
3 subsequent experiments. Successfully infected animals are then dosed by
4 oral gavage or as a provision of the test compound in diets with sulforaphane
5 or another compound as provided herein. To validate dosage, blood obtained
6 by cardiac puncture is processed for quantitative determination of
7 isothiocyanates and their dithiocarbamate metabolites in the serum or plasma
8 of a subset of animals (Ye *et al.*, *Clin Chem Acta* (2001) [in press]). Degree
9 of inflammation is assessed with the assistance of a pathologist and a
10 physician who are familiar with the appearance of gastric inflammation and
11 grade such gastritis using a modified Sydney system (CS Goodwin, *J*
12 *Gastroenterol Hepatol*, 6:235-237 (1991); XY Chen *et al.*, *J Clin Pathol*,
13 52:612-615 (1993)) and the 0-3 scale described by Lee *et al.*, *Zentralbl*
14 *Bakteriol*, 280:38-50 (1993), for acute inflammation, chronic inflammation
15 and atrophy.

16 If *in-vitro* activity is identified, therapy with Moringa tree leaves or
17 seeds, or broccoli or cauliflower sprouts or seeds, or extracts made from
18 these items can be useful to either ameliorate or cure peptic ulcers caused
19 by *H. pylori*. If there is anti-*H. pylori* antibiotic activity, therapy as indicated
20 above is also effective to prevent *H. pylori* infection and theoretically reduce
21 the incidence of stomach cancer which is related to *H. pylori* infection.

22 23 Example 4

24 *Helicobacter pylori* has been implicated as having a direct role in the
25 generation of oxidative stress in colonized gastric mucosal tissue. Shirin et
26 al. (Cancer Letters 164:127-133 (2001)) have demonstrated that
27 *Helicobacter pylori* causes a transient initial increase (1 h) in glutathione

(GSH) levels in cultured AGS cells, but that intracellular GSH stores were subsequently depleted completely after 24 h. They also showed that GSH concentrations in gastric mucosal from antral biopsies were significantly lower in *H. pylori* colonized human subjects (n = 19) than in normal controls (n = 38).

AGS cells are cultured in microtiter well plates and treated with concentrations of sulforaphane (SF) and 4-(α -L-rhamnopyranosyloxy)benzyl isothiocyanate (4RBITC) designed to induce QR levels several-fold above those of untreated controls, at 48 h. Low levels of bacteria *H. pylori* (Hp) are introduced to the plates at 1, 4, and 20 h post-induction. Quinone reductase (QR; a key Phase 2 detoxification and antioxidant enzyme) levels are assessed at both one and two days after induction. Cellular GSH and protein levels are determined at these time points.

Plate	Inducer (@24 h)	Hp Trtmnts (3/plate)*	Endpoint (QRIP, GSH, Protein)
1	untreated cntrl	25 h	48 h
2	untreated cntrl	28 h	48 h
3	untreated cntrl	44 h	48 h
4	untreated cntrl	25 h	72 h
5	untreated cntrl	28 h	72 h
6	untreated cntrl	44 h	72 h
7	SF (~ 20 uM)	25 h	48 h
8	SF (~ 20 uM)	28 h	48 h
9	SF (~ 20 uM)	44 h	48 h
10	SF (~ 20 uM)	25 h	72 h

1	11	SF (~ 20 uM)	28 h	72 h
2	12	SF (~ 20 uM)	44 h	72 h
3	13	4RBITC (~ 20 uM)	25 h	48 h
4	14	4RBITC (~ 20 uM)	28 h	48 h
5	15	4RBITC (~ 20 uM)	44 h	48 h
6	16	4RBITC (~ 20 uM)	25 h	72 h
7	17	4RBITC (~ 20 uM)	28 h	72 h
8	18	4RBITC (~ 20 uM)	44 h	72 h

9 *1-fresh medium; 2-fresh medium + *H. pylori*; 3-fresh medium + heat-killed
10 *H. pylori*

11 Example 5

12 An animal model of *H. pylori* infection is used to assess the efficacy of
13 glucosinolates, isothiocyanates, including sulforaphane, or derivatives thereof
14 to inhibit the growth of *H. pylori* in an *in vivo* setting. The animal model is
15 described in Lozniewski *et al.*, Infect Immun. 67(4): 1798-1805 (1999),
16 which is hereby incorporated by reference in its entirety. Briefly, human
17 embryonic stomachs are obtained after legal abortion and grafted onto nude
18 (or severe combined immunodeficient) mice, under the skin of the abdomen.
19 Eight days after implantation, the abdominal skin is reopened and gastric
20 juice from the fetal stomachs is aspirated, to check the acidity, and a
21 catheter is implanted into the grafted stomach. Subsequent to catheter
22 implantation, *H. pylori* is introduced into the grafted stomach, via the
23 catheter, and allowed to infect the tissue. At various time points after the
24 initial *H. pylori* inoculation, the infection is evaluated by testing the acidity of
25 the gastric juice and by histological evaluation of biopsies.

1 After successful infections are confirmed, the stomachs are dosed
2 with, for example, sulforaphane, through the catheter and infections are re-
3 evaluated at various time points to determine the efficacy of sulforaphane in
4 treating *H. pylori* infections.